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Protein chip technology

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Microarray technology has become a crucial tool for large-scale and high-throughput biology. It allows fast, easy and parallel detection of thousands of addressable elements in a single experiment. In the past few years, protein microarray technology has shown its great potential in basic research, diagnostics and drug discovery. It has been applied to analyse antibody–antigen, protein–protein, protein–nucleic-acid, protein–lipid and protein–small-molecule interactions, as well as enzyme–substrate interactions. Recent progress in the field of protein chips includes surface chemistry, capture molecule attachment, protein labeling and detection methods, high-throughput protein/antibody production, and applications to analyse entire proteomes.

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Abbreviations

AFM	atomic force microscope
GST	glutathione-*S*-transferase
PDMS	polydimethylsiloxane
PI	phosphatidylinositide
PVDF	poly(vinylidene fluoride)
SAM	self-assembled monolayer
SELDI	surface-enhanced laser desorption/ionization
SPR	surface plasmon resonance

Introduction

The past ten years have witnessed a fascinating growth in the field of large-scale and high-throughput biology, resulting in a new era of technology development and the collection and analysis of information. The challenges ahead are to elucidate the function of every encoded gene and protein in an organism and to understand the basic cellular events mediating complex processes and those causing diseases [1–4]. Miniaturized and parallel assay systems, especially microarray-based analyses, are crucial to large-scale and high-throughput biological analysis, as they are a rapid and economic way to interpret gene function [3,5,6], as demonstrated by DNA microarray approaches [7,8]. In a microarray format, capture molecules are immobilized in a very small area, and probed for various biochemical activities. High signal intensities and optimal signal-to-noise ratios can be achieved under ambient analyte conditions [3]. The microarray format has become the leading technology that enables fast, easy and parallel detection of thousands of addressable elements and side-by-side measurements.

Despite the success of DNA microarrays in gene expression profiling and mutation mapping, it is the activity of encoded proteins that directly manifest gene function. Thus, one would expect protein microarrays, in which proteins are prepared, arrayed and analysed at high spatial density, to be particularly powerful for analysing gene function, regulation and a variety of other applications. Proteins are more challenging to prepare for the microarray format than DNA, and protein functionality is often dependent on the state of proteins, such as post-translational modifications, partnership with other proteins, protein subcellular localization, and reversible covalent modifications (e.g. phosphorylation). Nonetheless, in recent years there have been considerable achievements in preparing microarrays containing over 100 proteins and even an entire proteome [1,2,9–11]. Alternative array formats have also been developed including tissue arrays [12], living cell arrays [13•,14•], peptide arrays [1,15–17,18••], antibody/antigen arrays [19••,20], protein arrays [21,22,23••–25••], carbohydrate arrays [26••,27••], and small-molecule arrays [28••]. However, technological challenges in the field of protein microarrays still remain.

In this review, we discuss recent progress in the field of protein chips, including surface chemistry, capture molecule attachment, protein labeling and detection methods, high-throughput protein/antibody production, and applications to analyse protein families and entire proteomes.

Manufacture of protein chips

It is important that protein chips retain proteins in an active state at high densities, are compatible with most commercial arrayers and scanners, and can be printed in such a fashion that the proteins remain in a moisturized environment. Soft substrates such as polystryrene, poly(vinylidene fluoride) (PVDF), and nitrocellulose membranes, which have been used to attach proteins in traditional biochemical analyses (e.g. immunoblot and phage display), are often not compatible for protein microarrays [2,16,22]. These surfaces often do not allow a suitable high protein density, the spotted material may spread on the surface, and/or they may not allow optimal signal to noise ratios [1,3,9,11]. Thus, most projects have

turned to using glass microscope slides or other materials that have been derivatized to attach proteins on their surface at high density. These slides have low fluorescence background and are compatible with most assays.

Different types of protein chips

A variety of types of chip have been designed, including 3D surface structures, nanowell and plain glass chips (Table 1). Polyacrylamide gel packet and agarose thin film microarrays, patterned by using photolithography technology on a glass surface, have been created by Guschin *et al.* [29] and Afanassiev *et al.* [30], respectively (Figure 1). Because both acrylamide and agarose form highly porous and hydrophilic matrixes, capture molecules, such as DNA, proteins and antibodies, can readily diffuse into the porous structure and are immobilized by cross-linking to the reactive ligands modified in the matrixes. Analytes are then added to these 3D arrays to carry out the biochemical assays [29]. Because of the formation of 3D matrixes on the glass surface, the capacity of protein immobilization is much higher than that on a 2D surface; the homogeneous water environment minimizes protein denaturing and thereby helps keep proteins in their active states. In addition to the sophisticated processes of creating such 3D matrixes, the major disadvantage of the 3D arrays is that it is more difficult to change buffers and recover trapped molecules from the matrix microarrays [4].

In contrast to 3D arrays, Zhu *et al.* [24••] fabricated an open structure, namely nanowells, on a polydimethylsiloxane (PDMS) surface supported by the standard glass slides. The nanowells significantly reduce evaporation and minimize cross-contamination and background. Because of the open nanowell structure, different components and buffers can be sequentially added, which is crucial for multiple-step biochemical assays. In addition, captured molecules can be easily recovered from the nanowells. When covered with gold in the nanowells, it is expected that high-throughput mass spectrometry and surface plasmon resonance (SPR) analyses can be performed. The biggest disadvantage of this technology it that specialized equipment is required to load the nanowells at high density.

Many groups now directly array proteins and antibodies/antigens onto plain glass surface [19••,20,23••,25••,31•,32]. To keep proteins in a wet environment during the printing process, high percent glycerol (30–40%) is used in sample buffer and the spotting is carried out in a humidity-controlled environment [23••,25••].

Surface chemistry

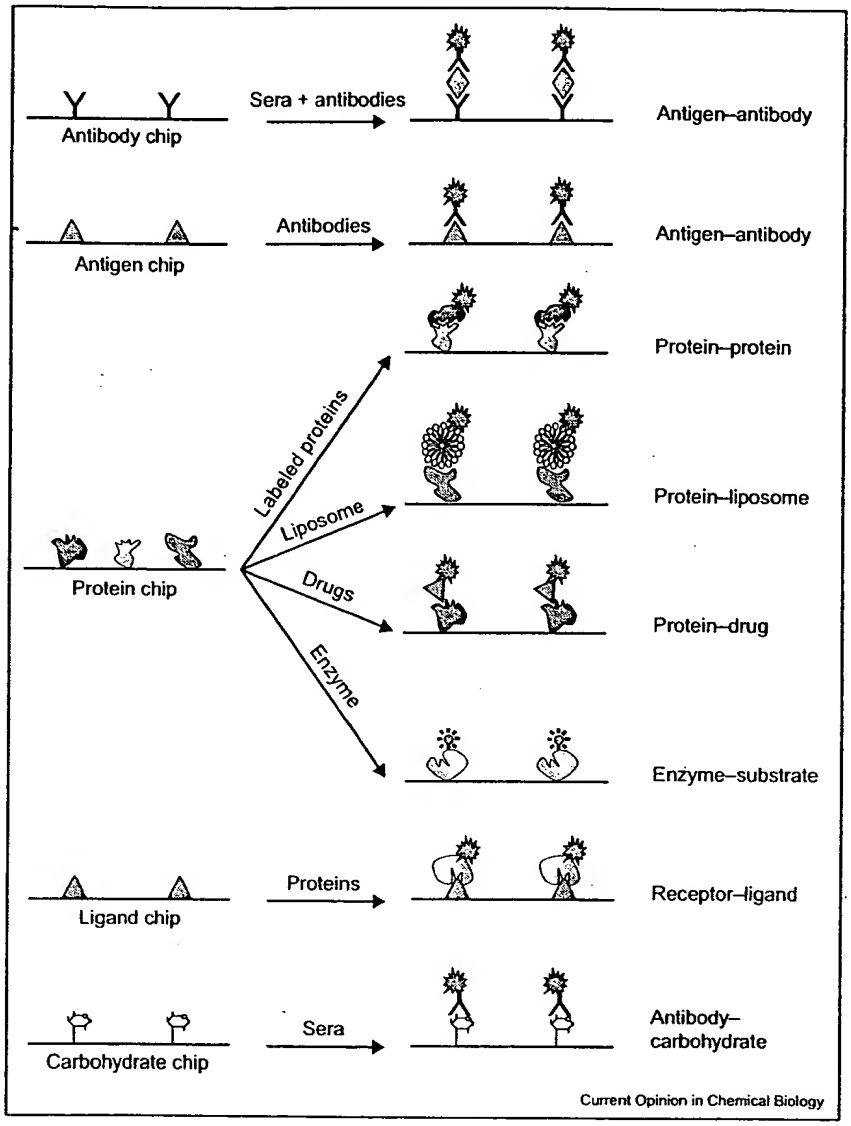
To attach proteins to a solid substrate, the surface of the substrate has to be modified to achieve the maximum binding capacity (Figure 2). A convenient method is to coat the glass surface with a thin nitrocellulose membrane or poly-L-lysine such that proteins can be passively adsorbed

Table 1

Comparison of current antibody/protein microarrays.

Surface	Attachment	Advantage	Disadvantage	References
PVDF	Adsorption and absorption	No protein modification requirement, high protein binding capacity	Non-specific protein attachment in random orientation	[2,16]
Nitrocellulose	Adsorption and absorption	No protein modification requirement, high protein binding capacity	Non-specific binding, high background, Low-density arrays	[20,22]
Poly-lysine coated	Adsorption	No protein modification requirement	Non-specific adsorption	[19•]
Aldehyde-activated	Covalent cross-linking	High-density and strong protein attachment, High-resolution detection methods available	Random orientation of surface-attached proteins	[23••,25••]
Epoxy-activated	Covalent cross-linking	High-density and strong protein attachment, High-resolution detection methods available	Random orientation of surface-attached proteins	[24••]
Avidin coated	Affinity binding	Strong, specific and high-density protein attachment, low-background	Proteins have to be biotinylated	[58]
Ni-NTA coated	Affinity binding	Strong, specific and high-density protein attachment, low-background, uniform orientation of surface attached proteins	Proteins have to be Hisx6 tagged	[25••]
Gold-coated silicon	Covalent cross-linking	Strong and high-density protein attachment, low-background. Can be easily coupled with SPR and mass spectrometry	Random orientation of surface attached proteins, tough to fabricate, not commercially available	[18••,35]
PDMS nanowell	Covalent cross-linking	Strong and high-density protein attachment, well suited for sophisticated biochemical analyses	Random orientation of surface attached proteins	[24••]
3D gel pad and agarose thin film	Diffusion	High protein binding capacity, no protein modification requirement	Tough to fabricate, not commercially available	[29,30]
DNA/RNA coated	Hybridization	Strong, specific and high-density protein attachment, low-background, uniform orientation of surface attached proteins	Sophisticated *in vitro* production of labeled proteins	[59]

Figure 1

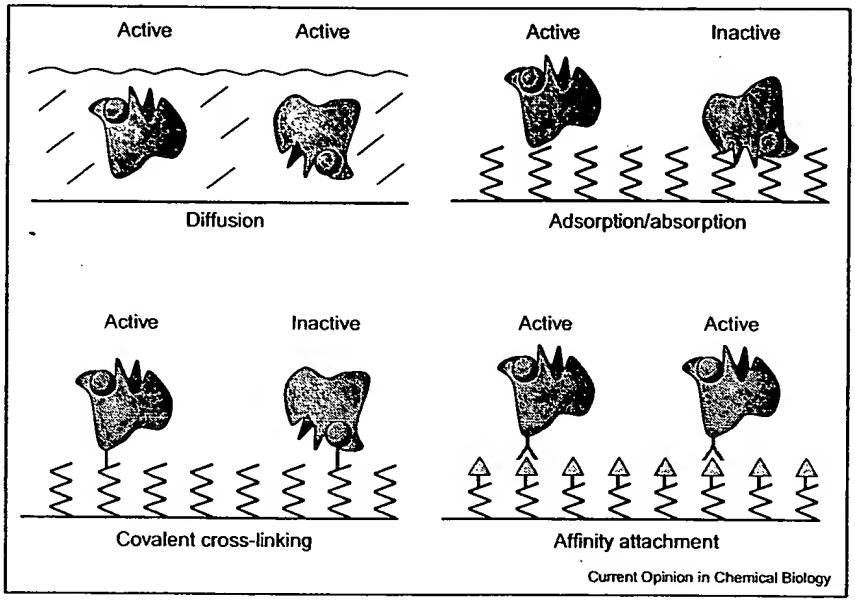


Applications of protein microarrays. There are two general types of protein microarray: analytical and functional protein microarrays. Analytical microarrays involve a high-density array of affinity reagents (e.g. antibodies or antigens) that are used for detecting proteins in a complex mixture. Functional protein chips are constructed by immobilizing large numbers of purified proteins on a solid surface. Unlike the antibody–antigen chips, protein chips have enormous potential in assaying for a wide range of biochemical activities (e.g. protein–protein, protein–lipid, protein–nucleic-acid, and enzyme–substrate interactions), as well as drug and drug target identification. Small molecule and carbohydrate microarrays are other types of analytical microarrays that have been demonstrated to be capable of studying protein binding activities to ligands and carbohydrates.

to the modified surface through non-specific interactions [20,22,33]. The attached proteins lay on the surface in random orientation and can be washed off under stringent washing conditions. In addition, the noise level is usually higher because of the non-specific adsorption/absorption.

To achieve more specific and stronger protein attachment, several groups have created reactive surfaces on glass that can covalently cross-link to proteins [23••–25••]. In general, a bifunctional silane cross-linker is used to form a self-assembled monolayer (SAM), which has one functional group that reacts with the hydroxyl groups on glass surface, and another free one that can either directly react with primary amine groups of proteins (i.e. aldehyde or epoxy groups) or can be further chemically modified to reach maximum specificity [28••,34]. Gold-coated glass surface is another variation [18••,35]. To form a SAM on gold surface, bifunctional thio-alkylene is usually used,

Figure 2



Comparison of different protein attachment methods. Proteins can be attached to various kinds of surface via diffusion, adsorption/absorption, covalent cross-linking and affinity interaction. Except affinity attachment, proteins are usually laid on the surface in a random fashion, which may alter the native conformation of proteins, reduce the activity of proteins, or make them inaccessible to probes. However, when proteins are attached to the surface via their affinity tags, it is very likely that every protein molecule uniformly attaches to the surface and, therefore, proteins are more likely to remain in their native conformation, while the analytes have easier access to the active sites (indicated by the red dots) of proteins.

which has a SH-group that reacts with gold, and another free one that reacts with capture molecules. The advantage of using gold-coated surface is that SPR and mass spectrometry can potentially be integrated as detection methods to monitor the dynamics of the reactions, or to identify the captured molecules, respectively [18••,35,36]. This approach provides the opportunity to study dynamics of biochemical reactions in a high-throughput fashion, and has great potential in drug and drug-target discovery and biomedical research [36].

In the above covalent cross-linking approaches, because the reactive ligands also exist in the side chains of proteins, it is plausible that the attached proteins attach to the surface in a random fashion, which may alter the native conformation of proteins, reduce the activity of proteins, or make them inaccessible to probes (Figure 2). Perhaps the best means of protein attachment is through highly specific affinity interactions [3,24••,25••]. Proteins fused with a high-affinity tag at their amino or carboxy terminus are linked to the surface of the chip via this tag, and hence, all of the attached proteins should orient uniformly away from the surface (Figure 2) [25••]. Using this method, immobilized proteins/antibodies are more likely to remain in their native conformation, while the analytes have easier access to the active sites of proteins. This approach was first successfully demonstrated in attaching 5800 fusion proteins containing a His tag onto a nickel-coated glass slide [25••]. It should also be possible to use other affinity methods such as glutathione/glutathione-*S*-transferase (GST) and phosphonate/serine esterase cutinase ligand/protein tags [37].

Protein delivery systems

Although a 96-format dot blot instrument has been used to create low-density protein arrays on filters [9,22,33], high-density protein microarrays (>30 000 spots per slide) can be achieved using robotic contact printing tools, such as those developed for creating DNA microarrays [23••,25••]. The contact printing arrayers deliver sub-nanoliter sample volume directly to the surface using tiny pins with or without capillary slots. Because these contact printing robots cannot align their pins to the pre-fabricated structures and need to touch the surface, non-contact robotic printers, which use ink-jet technology, were used to deposit nanoliter to picoliter protein droplets to polyacrylamide gel packets [21] and nanowells [24••]. Although the current Packard ink-jet microarrayer can be slowed when spotting many different samples and the shearing force during drop formation may damage some samples [1], it is not restricted to the surface structure and is well suited for more complicated biochemical assays. Recently, electrospray deposition technology was applied to deliver dry proteins to a dextran-grafted surface [38]. This technology further reduced the spot size from ~150 μm to ~30 μm.

Table 2

Summary of current detection methods used in protein microarray experiments

Detection	Probe labeling	Data acquirement	Real time	Resolution	References
ELISA	Enzyme-linked antibodies	CCD imaging	No	Low	[20,28••,38]
Isotropic labeling	Radio isotope-labeled analyte	X-ray film or phosphoimager	No	High	[22,23•,24••]
Sandwich immunoassay	Fluorescently labeled antibodies	Laser scanning	No	High	[20]
SPR	Not necessary	Refractive index change	Yes	Low	[41–44]
Non-contact AFM	Not necessary	Surface topological change	No	High	[40]
Planar waveguide	Fluorescently labeled antibodies	CCD imaging	Yes	High	[45]
SELDI	Not necessary	Mass spectrometry	No	Low	[39]
Electro-chemical	Metal-coupled analyte	Conductivity measurement	Yes	Medium	[60]

Probe detection methods

Fluorescence detection methods are generally the preferred detection method (Table 2) because they are simple, safe, extremely sensitive and can have very high resolution [1,3]. They are also compatible with standard microarray scanners. Typically, a chip is either directly probed with a fluorescent molecule (e.g. protein or small molecule) or in two step by first using a tagged probe (e.g. biotin), which can then be detected in a second step using a fluorescently labeled affinity reagent (e.g. streptavidin). Another fluorescent labeling method is rolling circle amplification (RCA), which is extremely sensitive [39]. However, other detection methods can also be used. For example, ELISA was first used to detect proteins for both filter arrays [20,40] and glass arrays [29]. Ge [22], and Zhu *et al.* [24••] have used radioisotope labeling to study protein–protein, protein–DNA, protein–drug interactions on filter arrays, and kinase–substrate interactions in nanowells, respectively.

Because labeling molecules can sometimes affect protein activity and are restricted to the available detection channels, non-labeling methods have advantages as a direct detection approach for antibody microarrays. SELDI (surface-enhanced laser desorption/ionization) mass spectrometry has been used to detect low-density arrays of captured proteins [41]. Captured proteins on an array of metal surface (SELDI protein array) were vapourized using a laser beam, followed by the analysis of mass spectrometry data to reveal the identities of these proteins. The atomic force microscopy (AFM) method takes advantage of surface topological changes to identify the captured proteins on an antibody array [42]. When the immobilized rabbit IgG on a gold surface bound to its complimentary antibodies, goat ant-rabbit IgG, AFM could detect the height increase, and therefore, revealed the binding activities.

To study the kinetics of antigen–antibody interactions, however, real-time detection methods will be useful. SPR has matured as a versatile detection tool to study the kinetics of receptor–ligand interactions with a wide range of molecular weights, affinities and binding rates [43–45]. Although the commercially available SPR chips are limited to a few channels, Myszka and Rich [46] described a sensor surface with 64 individual immobilization sites in a single flow cell. Alternatively, Sapsford *et al.* [47] developed an antibody array biosenor to study the kinetics of antigen binding using a planar waveguide as the detection method. More importantly, they demonstrated that significant signal intensity could be achieved from spots as small as 200 μm in diameter. It is therefore expected that the latter approach is well suited for high-throughput and parallel kinetics studies.

Two functional classes of protein microarrays

There are two general types of protein microarrays. Firstly, analytical microarrays in which antibodies, antibody mimics or other proteins are arrayed and used to measure the presence and concentrations of proteins in a complex mixtures. Secondly, functional protein microarrays, in which sets of proteins or even an entire proteome are prepared and arrayed for a wide range of biochemical activities.

Analytical microarrays

Analytical microarrays involve a high density array of affinity reagents that are used for detecting proteins in a complex mixture. They have enormous potential for monitoring protein expression on a large-scale, a process that is sometimes termed protein profiling.

Antibody microarrays

The most common form of analytical arrays are antibodies/antibody mimic arrays in which antibodies (or similar reagents) that bind specific antigens are arrayed on a glass slide at high density. A lysate is passed over the array and the bound antigen is detected after washing. Detection is usually carried out by using labeled lysates or using a second antibody that recognizes the antigen of interest. The biggest challenge with these methods is producing reagents that identify the protein of interest and with high enough specificity in a high-throughput fashion.

Antibodies are the traditional reagent of choice for detecting proteins in complex mixtures. However, polyclonal sera are often not specific and are expensive to produce,

and the conventional hybridoma method of producing highly specific monoclonal antibodies is also time-consuming, laborious and costly. Recently, alternative methods, such as phage antibody-display, ribosome display, SELEX (systematic evolution of ligands by exponential enrichment), mRNA display, and affibody display, have been developed to expedite the production of antibodies and/or antibody mimics [1–3,9]. All of these approaches involve the construction of large repertoires of viable regions with potential binding activity, which are then selected by multiple rounds of affinity purifications. The binding affinity of the resulting candidate clones can be further improved using maturation strategies. However, the ideal selection system, which is not only fast, robust, sensitive, and of low cost, but automated and minimized, is yet to be fully developed [3,9].

In spite of the challenge in obtaining specific antibodies, several studies using antibodies have recently appeared. In one of the largest studies to date, Sreekumar *et al.* [31•] spotted 146 distinct antibodies on glass to monitor the alternations of protein quantity in LoVo colon carcinoma cells. Their results revealed radiation-induced up-regulation of many interesting proteins, including p53, DNA fragmentation factor 40 and 45, tumour necrosis factor-related ligand, as well as down-regulated proteins.

The most significant problem with antibody arrays is specificity. Proteins are often present in a very large dynamic range (10^6); thus, reagents that might have high affinity for one protein, but are low affinity for another will still exhibit detection of the lower affinity protein if it is much more prevalent. Haab *et al.* [19••] have investigated the ability of 115 well-characterized antibody–antigen pairs to react in high-density microarrays on modified glass slides. 30% of the pairs showed the expected linear relationships, indicating that a fraction of the antibodies were suitable for quantitative analysis. To avoid this problem, many groups have turned to using sandwich assays, in which the first antibody is spotted on the array and then the antigen is detected with a second antibody that recognizes a different part of the proteins. This approach dramatically increases the specificity of the antigen detection, but required that a least two high-quality antibodies exist for each antigen to be detected.

Other analytical microarrays

In addition to antibody microarrays, other analytical microarrays have been developed. These include microarrays for profiling antibodies in a patient's serum, essentially the reciprocal of that described above. Joos and colleagues [20] used 18 diagnostic markers for autoimmune diseases to form an autogen microarray and screened for antigen–antibody interactions. Hiller *et al.* [48] arrayed 94 purified allergen molecules, which included most common allergen sources, on glass slides to miniaturize the allergy test. These allergen molecules were not restricted to proteins, but also included peptides and small molecules. The allergen microarrays were specifically used to determine and monitor allergic patients' IgE reactivity profiles to large numbers of disease-causing allergens in single measurements. Only minute amounts of serum were required. Potential new leads to allergic diseases were revealed, and some of them have been confirmed using the traditional skin tests. To characterize autoantibody responses, Robinson *et al.* [49] robotically arrayed hundreds of autoantigens, including proteins, peptides, and other biomolecules, in eight distinct human autoimmune diseases onto glass slides to form the autoantigen microarrays. These arrays were incubated with patient serum samples to define the pathogenesis of autoantibody responses in human autoimmune diseases. To explore the possibility of quantitative measurement of serum-specific IgE using protein chip format, Kim *et al.* [50] used purified dermatophagoides pteronyssinus (Dp)-specific IgE to detect allergens in serum challenged with Dp, egg white, milk, soybean and wheat. These authors were able to demonstrate that quantitative measurement of allergen in a protein mixture could be achieved.

Functional protein chips

Functional protein chips are constructed by immobilizing large numbers of purified proteins on a solid surface. Unlike the antibody chips, which are mainly developed for diagnostics and profiling of protein and epitope expression, protein chips have enormous potential in basic research, as well as drug and drug target identification (Figure 1). For example, both the Mrksich [18••] and Schreiber [23••] groups have demonstrated the potential of using protein microarrays to conduct enzymatic assays to identify downstream targets of kinases. However, the first great obstacle to overcome is the purification of large numbers of proteins in a high-throughput manner.

High-throughput protein production

To analyse the biochemical activities of as many proteins as possible, many research groups and companies have contributed tremendous effort in developing high-throughput protein purification methods. The combination of recombinant proteins and affinity purification has been used to purify proteins from various host cells, including lines from *Escherichia coli*, yeast, insects and humans [9,25••,40,51,52].

Leuking *et al.* [11] cloned cDNAs from human fetal brain tissues as C-terminal Hisx6-tagged fusions. The Hisx6 tags were used first as an indicator of in-frame fusion proteins and then served as an affinity tag for high-throughput protein purification from *E. coli*. In a later report, LaBaer and colleagues [51] created a system (FLEXP) that performs from cDNA cloning to protein production from *E. coli* in a fully automated fashion. In a test case, ~80% of 336 random cDNA clones could

successfully purify fusion proteins in full length. Because the purification process was automated, at least 1000 proteins could be purified in one day. However, because eukaryotic proteins expressed in prokaryotic systems are not post-translationally modified, our group has developed a high-throughout protein purification method from the budding yeast [25••]. The yeast genes were cloned as N-terminal GST:Hisx6 fusions, and purified using the GST affinity tags. In two weeks, >6500 yeast proteins could be purified individually from 3 ml culture. For the same reason, Albala *et al.* [52] chose 72 unique human cDNA clones to create an array of recombinant baculoviruses, from which 42% of the clones produced soluble fusion proteins in a 96-well format.

Alternatively, proteins can be produced using cell-free expression systems. For example, Keefe and Szostak [53] established a mRNA display system, in which each protein was *in vitro* translated and covalently linked through its carboxy terminus to the 3′ end of its coding mRNA. More interestingly, He and Taussig [54] created a protein *in situ* array (PISA), which combines the protein production and immobilization in one step. Although the experiment was performed in microtiter dishes, it is plausible that the system can be easily automated and applied to a microarray format.

Applications of functional protein chips

Functional protein chips like traditional assays performed in microtiter plates [55] are suitable for a wide variety of biochemical analyses. Unlike microtiter plates, however, they are much more amenable to high-throughput studies and use small amounts of reagents. In early proof-of-concept studies, MacBeath and Schreiber [23••] fabricated protein microarrays with three purified proteins at high density, and performed protein–protein, protein–ligand, and kinase–substrate interactions using three test systems. Likewise, Mirzabekov and co-workers [29] demonstrated that proteins immobilized in the gel pads could still show their enzymatic activities.

Studies analysing large sets of proteins have recently been performed. Using a PDMS nanowell chip mounted on glass slides, Zhu *et al.* [24••] analysed the activity of 119 yeast kinases for 17 different substrates. The substrates were first covalently immobilized to individual nanowells, and individual protein kinases with radio-labeled ATP were incubated with the substrates. After washing away the kinases and unincorporated ATP, the nanowell chips were analysed for phosphorylated substrates using a phosphoimager. Not only known kinase–substrate interactions were identified, but also many novel activities were revealed. This included the unexpected discovery that one-fourth of yeast protein kinases are capable of phosphorylating their substrates on tyrosine, even though the kinases are members of the Ser–Thr family of protein kinases.

Because the ultimate goal of proteomics is to study biochemical activities of every protein encoded by an organism, Zhu *et al.* [25••] prepared the first proteome chip. They cloned ~94% (>5800 of 6200) of the yeast open reading frames in a yeast expression vector that expresses the proteins as N-terminal GST-Hisx6 double tagged fusions and developed a high-throughput yeast protein purification method to individually purify proteins. 80% of yeast proteins are full length and of sufficient quantities that they are detectable by most assays. The proteins were purified using the GST tags and were then attached to Ni-NTA-coated glass slides using the HisX6 tags. In our initial study, the chips were probed with Cy3-labeled calmodulin and various phosphatidylinositides (PIs). Calmodulin is a highly conserved calcium-binding protein that regulates many signaling pathways and has many known binding partners. In addition to identifying known interactions, 33 novel binding proteins were detected. Sequence comparison revealed a novel binding motif that was related, but distinct from, the previous known calmodulin-binding motif. To demonstrate that proteome chips could be used to globally probe for novel activities, the chips were incubated with five different PIs, which are important secondary messengers that regulate diverse cellular processes [56]. 150 novel lipid-binding proteins were identified, 49 of which exhibited preferential binding to PIs. These results convincingly showed that proteins immobilized on a surface were able to bind to low molecular weight compounds. This suggests that an entire proteome can be immobilized on a glass surface to directly screen for interactions with proteins and small molecules.

Peptide arrays

It is of great interest and importance to identify epitopes in proteins that define the core activity. To study the substrates of the nonreceptor tyrosine kinase *c-Src*, Houseman *et al.* [18••] immobilized 9-mer peptide substrates on a gold-coated glass surface to form a high-density peptide microarray, and characterized the phosphorylation of the peptide using SPR, fluorescence and phosphoimaging. They could also quantitatively evaluate the effect of three known inhibitors of the kinase. Although their work was still primitive, the authors demonstrated the potentials of coupling peptide chips with various detection methods to quantitatively study dynamics of enzyme–substrate interactions, and applications in drug discovery. Our group has also designed 20 17-mer peptide substrates and covalently immobilized them to epoxy-activated glass surface (unpublished data). 120 yeast kinases were screened for their preferred substrates.

Because peptides are much shorter and more stable than proteins, high-density peptide microarrays can be fabricated by direct synthesis of peptides on a surface using photolithography or light-directed synthesis [15,57].

These approaches greatly save cost in peptide synthesis because only a tiny amount of material is needed.

Conclusion

Protein microarrays are poised to become one of the most powerful tools in the field of large-scale biology because of their enormous potential in basic research, diagnostics and drug discovery. High-density robotically spotted protein microarrays on glass have been validated to analyse an entire proteome and hold great promise for high-throughput discovery applications [19••,23••,25••]. Improvements in generating large sets of antibody reagents, recombinant proteins from a variety of host cells and other types of capture molecule will further increase the interest in this field.

The fast growth of protein microarray technologies is fueled by the continuous growth of genomic information. The actual impact of these new technologies for proteomic and medical research, drug discovery and clinical diagnostics are yet to be fully realized. It is expected that the integration of large datasets from different approaches will result in the generation of a huge network and deepen our understanding of the molecular mechanisms of life.

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